immune system activation. Alterations in the regulation of CaN can lead to disorders such as Down syndrome-related mental retardation and cardiac hypertrophy. CaN is also the target for the immunosuppressant drugs FK506 and cyclosporin A. The regulation of CaN function is not well understood at the molecular level. CaN is inactive until bound by calmodulin (CaM). CaM binds at a site towards the N-terminus of a 95 residue regulatory domain in CaN. This regulatory domain is believed to be disordered. The binding of CaM to CaN causes an autoinhibitory domain located C-terminal to the regulatory domain to be ejected from CaN's active site. We hypothesize that the CaN regulatory domain undergoes a folding transition upon CaM binding, and that this folding provides the driving force for pulling the autoinhibitory domain from the active site. We have made a fragment of CaN that consists of the regulatory domain, autoinhibitory domain and a short C-terminal domain. We will present data from CD spectroscopy, fluorescence, NMR and analytical ultracentrifugation experiments that indicate this fragment is largely disordered in the absence of CaM, and gains structure when CaM binds.

1135-Plat
Bioinformatic Analysis of the Role of Intrinsic Disorder in Multiple Specificity
Christopher J. Oldfield, Vladimir N. Uversky, A. Keith Dunker.
School of Informatics, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA.
Several lines of evidence suggest that intrinsically disordered proteins (IDPs) are a common mechanism used by nature to mediate protein-protein interactions. IDPs lack a stable three-dimensional structure under physiological conditions and many such proteins have been characterized by several biophysical methods. Additionally, IDPs are estimated to be abundant within various proteomes, particularly eukaryotes, and carry out a variety of molecular functions without the prerequisite of a specific, stable structure. It is thought that IDPs can facilitate protein interactions through an ability to mediate binding diversity, where one of the proposed mechanisms for this is multiple specificity - i.e. recognition of multiple molecular partners through use of the same binding residues - through contextual folding of IDPs.
In previous work, two contrasting examples of proteins with multiple binding specificity were examined, 14-3-3ζ and p53, which exemplify the potential of intrinsic disorder for mediating protein interactions. 14-3-3ζ has a structured domain with a single binging pocket that is responsible for the binding of various protein partners through interaction with sequence divergent intrinsically disordered segments in these partners. In contrast, the disordered termini of p53 contain discrete regions that are each involved in many interactions with different protein partners, where these interactions carry out and regulate p53 function. The common theme in both of these examples is structural variability in the bound state that is enabled by intrinsic disorder in one of the partners in the unbound state.
In current work, the previous analysis is expanded to many other examples of proteins that interact with multiple partners using a common binding site. These data support the conjecture that intrinsic disorder enables binding to multiple partners and provides detailed information about induced fit in structured regions.

1136-Plat
High-throughput Characterization of Intrinsically Disordered Proteins from the Protein Structure Initiative
Derrick E. Johnson, Megan D. Sickmeier, Jingwei Meng, Tanguy Le Gall, Vladimir Uversky, A. Keith Dunker.
Indiana University School of Medicine, Indianapolis, IN, USA.
The identification of intrinsically disordered proteins (IDPs) among the targets that fail to form satisfactory crystal structures in the Protein Structure Initiative represent a key to reducing the costs and time for determining three-dimensional structures of proteins. To help in this endeavor, several Protein Structure Initiative Centers were asked to send samples of both crystallizable proteins and proteins that failed to crystallize. Initially, the abundance of intrinsic disorder in these proteins was evaluated via computational analysis using Predictors of Natural Disordered Regions (PONDR®) and the potential cleavage sites and corresponding fragments were determined. Then, the target proteins were analyzed for intrinsic disorder by their resistance to limited proteolysis. The rates of tryptic digestion of sample target proteins were compared to those of myoglobin, apomyoglobin and α-casein as standards of ordered, partially disordered and completely disordered proteins, respectively. Results from these digestion experiments generally correlated with the results of disorder predictions. At the next stage, the protein samples were subjected to both far-UV and near-UV circular dichroism (CD) analysis. For most of the samples, a good agreement between CD data, predictions of disorder and the rates of limited tryptic digestion was established. Most samples corresponded to proteins that were predicted to be ordered had slower digestion rates and showed a good amount of ordered structure as determined by near- and far-UV CD analysis. On the contrary, predicted to be disordered proteins were digested fast and possessed spectral features characteristic of IDPs. Further experimentation is being performed on a smaller subset of these samples in order to obtain more detailed information about the ordered/disordered nature of the proteins.

1137-Plat
How Does Charge Content Modulate Conformational Equilibria of Intrinsically Disordered Proteins? An Illustration Using Protamines
Albert Mao, Andreas Vitalis, Rohit V. Pappu.
Washington University in St. Louis, St. Louis, MO, USA.
Intrinsically disordered proteins (IDPs) adopt heterogeneous ensembles of conformations at equilibrium under physiological conditions. Just as the structure of a folded protein determines its function, the conformational ensemble of an IDP governs its interactions with binding partners. We seek quantitative descriptions of conformational equilibria anchored in polymer physics concepts that capture the richness of IDP phase diagrams. Recent studies by our lab showed that archetypal polar homopolymer IDPs favor collapsed ensembles in water despite the absence of hydrophobes, a counterintuitive result that even held for polypeptide backbones alone. We now turn our attention to highly charged peptides, which constitute a different archetype of IDP. We simulated a variety of protamines - a class of arginine-rich IDPs involved in the condensation of nuclear chromatin during spermatogenesis - in aqueous 125 mM salt solutions in order to elucidate the influence of charge content on conformational equilibria. The simulations were performed with ABSINTH, a Monte Carlo engine that employs our recently-developed implicit solvation model. We find that protamines with high charge asymmetry are similar in their adoption of extended bent-rod conformations, a result in agreement with theoretical predictions. Sequences with identical charge asymmetry but different charge composition exhibited similar characteristics in terms of overall size measures such as radius of gyration. However, local properties such as alpha helix propensity remained strongly dependent on the particular sequence. These findings point towards a possible engineering principle for IDP sequence design: general size requirements set the charge asymmetry, while local conformational specifications govern the particular sequence. This principle is consistent with the evolutionary pattern of protamines: while sequences exhibit hypervariability across species, arginine content is highly conserved.
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1138-Plat
Characterization of the Unfolded State Under Native Conditions: A Missing Piece of the Protein Folding Puzzle
Daniel P. Raleigh, Bing Shan, Wenli Meng, Jae-Hyun Cho, Humeyra Taskent.
State University of New York, Stony Brook, NY, USA.
The nature of the denatured state ensemble is controversial owing in large part to the difficulty of characterizing the structure and energetics of denatured state interactions. Denatured states can be populated under a variety of extreme conditions but the state which is most relevant for protein folding and engineering is the denatured state ensemble which is populated in the absence of denaturant under native conditions. Unfortunately this state is usually experimentally inaccessible. We reported detailed characterized of the denatured state populated under native conditions for two α-β proteins, the N-terminal domain of the ribosomal protein L9 (NTL9) and the C-terminal domain of the same protein (CTL9) , as well as for a rapid folding all helical structure the villin headpiece helical subdomain, HP-36. Conditions have been found where the native and denatured states of CTL9 are both populated in the absence of denaturant and 1H, 15N and 13C NMR was used to define the conformation propensities of the denatured state. For NTL9 the thermodynamic linkage between proton binding and protein stability was used to characterize denatured state electrostatic interactions. Peptide models were exploited to characterize the denatured state of HP-36. In all three cases, the denatured states contains significant structure. The impact of this preformed structure on the kinetics and mechanism of protein folding is discussed.

1139-Plat
Distribution of Conformations Sampled by the Central Amino Acid Residue in GXG Peptides Inferred from Amide 1′ Band Profiles and NMR Scalar Coupling Constants
Andrew Hagarman[1], Thomas Measey[1], Daniel Matthieu[2], Harald Schwalbe[2], Reinhard Schweitzer-Stenner[1].
[1]Drexel University, Philadelphia, PA, USA, [2]Johann Wolfgang Goethe Universität, Frankfurt, Germany.

The conformational propensity of amino acids in the unfolded state of peptides and proteins is the subject of ongoing deliberation. Recent research has mostly focused on alanine, owing to its abundance in proteins and its relevance for the understanding of helix coil transitions. We have analyzed the amide I' band profiles of the IR, isotropic and anisotropic Raman, and VCD profiles of a series of GXG peptides, X representing a subset of the naturally occurring amino acids, in terms of a conformational model which explicitly considers the entire ensemble of possible conformations rather than representative structures. The distribution function utilized for satisfactory simulations of the amide I' band profiles was found to also reproduce a set of seven J-coupling constants reported by Graf et al[1]. The results of our analysis reveal a PPII fraction of 0.91 for the central alanine residue in GAG, which strongly corroborates the notion that alanine has a very high PPII propensity. We performed a similar analysis for X=E, F, S, V, K, L and M. Preliminary indication is that E, F, K and L exhibit a substantial PPII propensity, whereas S, V and M exhibit a less pronounced PPII propensity with an increased propensity for β-strand. We also used distributions from coil libraries and MD simulations to model amide I band profiles and J-coupling constants for alanine and valine. We found most of them to be inconsistent with our experimental data. Thus, these results clearly demonstrate that caution must be taken in using coil libraries and MD simulations to describe the unfolded state of peptides and proteins, and that experimental data are a prerequisite for quantitative evaluation of amino acid residues.

1140-Plat

Intrinsic Disorder and the Evolution of Viral Overlapping Genes

Pedro R. Romero[1], Corinne Rancurel[2], Mahvash Khosravi[1], A. Keith Dunker[1], David Karlin[3].
[1]Indiana University = Purdue University Indianapolis, Indianapolis, IN, USA, [2]Architecture et Fonction des Macromolcules Biologiques, Marseille, France, [3]Tous Chercheurs, Inmed, Marseille, France.

Overlapping genes result from frameshifts over pre-existing genetic sequences by a process called overprinting, yielding two or more different proteins encoded by the same nucleotides. In this situation, single base changes would frequently alter the amino acids in two or more proteins simultaneously, but such mutations would need to fit two or more sets of structural constraints. One way around such double or multiple constraints would be for overlapping genes to be enriched in structural disorder, which is more tolerant of mutations than is structure. To test this idea, we predicted structure/disorder in the protein products of manually curated overlapping genes from unspliced RNA viruses. Overlapping regions were found to be significantly more disordered than non-overlapping regions and to have a sequence composition biased towards disorder-promoting amino acids. For a subset of the overlapping genes in our dataset, the genetic sequences that were created *de novo* by overprinting of ancestral genes were determined. Most of the protein products of these novel genes are disordered and have unusual amino acid compositions. Furthermore, almost all of these gene products are accessory proteins rather than replicases or other proteins fundamental to viral replication or structure, and these proteins are orphans without homologues. Proteins that have been created by overprinting different homologues of the same genes display a diversity of functional and structural features, facts that are consistent with their *de novo* origin. Our results offer a glimpse of the structural and functional characteristics of protein regions encoded by genes created *de novo* by overprinting events in viruses. In most cases, intrinsically disordered gene products seem to help alleviate both the difficulty of generating structured proteins *de novo*, and the increased evolutionary constraints expected for multiple-coding genetic sequences.

Platform Y: Voltage-gated Ca Channels

1141-Plat

Persistent Increases In Ca^{2+} Influx Through Cav1.2 (I_{CaL})Induce Cardiac Conduction Disturbances And Sarcoplasmic Reticulum Ca^{2+} Overload To Induce Cardiac Arrhythmia And Sudden Death

Xiongwen Chen[1], Xiaoying Zhang[2], Mingxin Tang[1], David Harris[3], Yingxin Li[1], Hiroyuki Nakayama[4], Hongyu Zhang[1], Remus Berretta[1], Andrea Eckhart[3], Walter Koch[3], Jeffrey Molkentin[5], Steven Houser[2].
[1]Temple univ, Philadelphia, PA, USA, [2]Temple University, Philadelphia, PA, USA, [3]Thomas Jefferson University, Philadelphia, PA, USA, [4]Cincinnati Children's Hosp, Cincinnati, OH, USA, [5]Cincinnati Children's Hospital, Cincinnati, OH, USA.

Increases in Ca^{2+} influx through Cav1.2 has been observed in cardiovascular disease (CVD) and associated with cardiac arrhythmias. **Methods**: To mimic the enhanced Cav1.2 activity in CVD, we overexpressed the Cav1.2 β2a subunit in a transgenic mouse model. In-vivo ECGs, ion currents and intracellular Ca^{2+} were measured in transgenic (TG) and control (CTR) mice. **Results**: I_{CaL} was greater in TG myocytes (23.9 ± 2.5pA/pF, CTR 13.8 ± 1.6pA/pF). TG mice had enhanced cardiac performance (EF: TG 72.7 ± 1.3%, CTR 66.7 ± 1.5%) but died suddenly (TG 50% vs CTR 100% alive at 6 months), suggesting cardiac arrhythmias. In conscious mice, there was no difference in heart rate in CTR (571 ± 29bpm) and TG (541 ± 24bpm) mice but the QT interval was significantly shorter in TG (44.0 ± 5.5ms, CTR 58.2 ± 3.4ms) mice. Second degree AV block and ectopic premature ventricular beats were observed in all 4 TG mice but not in CTR mice. In anesthetized mice, there was no difference in heart rate (CTR 513 ± 20bpm, TG 526 ± 13bpm) but the PR interval (CTR 32.4 ± 1.4ms, TG 49.8 ± 6.2ms) and QRS duration (CTR 11.4 ± 0.8ms, TG 14.5 ± 0.8ms) were significantly prolonged in TG mice, indicating conduction defects. A significantly greater % of TG myocytes (28.5%) had early (EADs) and delayed (DADs) afterdepolarizations than CTR (0.0%) due to enhanced SR load (caffeine spritz and Fluo-4 F/F_0: TG 4.7 ± 0.4 vs. CTR 3.2 ± 0.3) and I_{NCX} (TG 2.15 ± 0.6pA/pF vs CTR 1.12 ± 0.3pA/pF at +60mV). However, action potential duration (APD) was significantly shorter in TG myocytes (APD90%: 40.0 ± 5.7ms vs. CTR: 100.6 ± 15.2ms) resulting from an increase of Ito (TG vs. CTR: 60.2 ± 0.8pA/pF vs. 18.7 ± 3.0pA/pF at +50mV). **Conclusion**: Persistent increases in Ca^{2+} influx through Cav1.2 cause both conduction disturbances and SR Ca overload, and induce cardiac arrhythmias with shortened APDs and QT intervals.

1142-Plat

Rem Selectively Abolishes β1-adrenergic Regulation Of $Ca_V1.2$ Channels In Heart

Xianghua Xu, Henry M. Colecraft.
Columbia University, New York, NY, USA.

β-adrenergic modulation of cardiac $Ca_V1.2$ channels is critical for sympathetic regulation of the heartbeat, and its disruption is a harmful hallmark of heart failure. RGK (Rem, Rem2, Rad, Gem/Kir) GTPases potently inhibit Ca_V channels by interacting with their auxiliary β subunits. Intriguingly, RGK proteins are present in heart, and their levels are elevated in heart failure. We examined the impact of the RGK GTPase, Rem, on $Ca_V1.2$ channels in heart cells and assessed whether there was crosstalk with the β-adrenergic modulation of the channel. Cultured adult guinea pig ventricular myocytes expressed robust $Ca_V1.2$ currents ($I_{Ca,L}$) (15.08 pA/pF) and responded to β1-adrenergic activation (1 μM isoproterenol + 1 μM ICI118,551) with a sharp, three-fold increase in current density. Isochronal cardiac cells expressing YFP-Rem, achieved through adenovirus infection, displayed a markedly lower basal current density (5.85 pA/pF). Nevertheless, the effect of Rem in heart is quantitatively smaller than seen in recombinant channels expressed in HEK 293 cells, which feature a virtual ablation of $I_{Ca,L}$. Surprisingly, the remaining Rem-insensitive $I_{Ca,L}$ in guinea pig heart cells was essentially unresponsive to β1-adrenergic stimulation. This was not due to disruption of the signaling pathway because isoproterenol-mediated increase in cardiac I_{Ks} remained unchanged. Intriguingly, the Rem insensitive $I_{Ca,L}$ remained responsive to forskolin. These results reveal an unexpected crosstalk between RGK GTPases and β-adrenergic signaling pathway at the level of cardiac $I_{Ca,L}$, and suggests that Rem selectively inhibits spatially distinct $Ca_V1.2$ channels in single heart cells.

1143-Plat

The Timothy Syndrome Mutation In Cav1.2 Causes Dendritic Retraction Through Calcium-independent Activation Of The Rhoa Pathway

Jocelyn F. Krey, **Ricardo Dolmetsch**.
Stanford University, Stanford, CA, USA.

L-type voltage-gated calcium channels (LTCs) play a key role in neuronal development by activating signaling pathways that regulate neuronal gene expression and morphology. A point mutation in the LTC CaV1.2, which blocks voltage-dependent inactivation (VDI), causes autism in Timothy Syndrome (TS) patients. While it is known how VDI influences the current through LTCs, it is not known how alterations in VDI affect the signalling function of CaV1.2 in neurons and ultimately cause developmental defects that lead to autism. Here we show that CaV1.2 channels containing the TS mutation (TS-CaV1.2) cause dendrite retraction and reduced dendrite branching in cortical neurons in vitro and in vivo. Surprisingly, we found that TS-CaV1.2 causes dendritic retraction independently of Ca2+ influx through the channel suggesting that the voltage-dependent conformational changes associated with VDI play an important and unexpected role in CaV1.2 signaling. In addition, we found that TS-CaV1.2 causes dendrite retraction by activating the RhoA signalling pathway. We found that the small GTP-binding protein Gem with the channel beta subunit play a critical role in mediating the calcium-independent activation of RhoA by TS-CaV1.2. Our results provide new insight